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Comparative study of anti-arthritic activity of methanolic extract of *Breynia retusa* and *Aglaonema hookerianum* leaves

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Abstract

Objective: To evaluate the comparative study of anti-arthritic activity of methanolic extracts of *Breynia retusa* (Euphorbiaceae) and *Aglaonema hookerianum* (Araceae) leaves.

Methods: Anti-denaturation method was performed by using bovine serum albumin (BSA) to evaluate the anti-arthritic potential.

Results: Methanolic extracts of *Breynia retusa* and *Aglaonema hookerianum* exhibited the presence of significant anti-arthritic activity. Here the extracts showed 75.86% and 81.43% of protein denaturation at highest conc. (1000ug/ml) and 33.87% and 43.54% at lowest conc. (31.25ug/ml), whereas the standard drug showed the 92.77% at 1000ug/ml and 52.15% at 31.25ug/ml conc.

Conclusion: These result suggested that both methanolic extract of *Breynia retusa* and *Aglaonema hookerianum* possess promising anti-arthritic activity

Keywords: *Breynia retusa*, *Aglaonema hookerianum*, anti-arthritic, inhibition, protein denaturation.

1. Introduction

Breynia retusa is a shrub with spreading branches. It grows generally in Bangladesh^[1], China, Bhutan, Cambodia, India, Laos, Malaysia, Nepal, Sri Lanka, Thailand, Vietnam^[2]. In Bangladesh, it is widely distributed in scrub forests of Sylhet and Chittagong Hill Tracts. The plant is used as astringent to the bowels and also useful in inflammations and diseases of the blood. The juice of the stem is used in conjunctivitis. Leaves employed to hasten suppuration^[1]. Another promising medicinal plant is *Aglaonema hookerianum* which is a herb. It is distributed in Bangladesh, North eastern India and Myanmar. In Bangladesh, it is found in the forests of Sylhet, Chittagong and Chittagong Hill Tracts. Vernacular name of this plant is known as *Chekhov*, *Khaichcha Parabol*, *Meggey* (Marma); *Hatharikhietok* (Tripura), *Lykho* (Khumi) and *Gach Petic*, *Shakkosala*, *Sikkachalal* (Chakma). The species is also used in the treatment of cirrhosis, flatulence, hyper acidity (gastritis) and tetanus^[3] and conjunctivitis and constipation^[4]. Extracted juice of spathe of this plant is used for stomachache by various tribes such as Khumi, Marma and Tripura^[5].

2. Materials and Methods

2.1 Plant material

Breynia retusa and *Aglaonema hookerianum* leaves were collected from a local area (Batali Hill and Naikkhongchari, Bandarban) of Chittagong division, Bangladesh and authenticated by the Botanist Dr. Shaikh Bokhtear Uddin, Assistant Professor, Department of Botany, University of Chittagong, Bangladesh.

2.2 Preparation of extract

The leaves were sun dried and ground. The ground (300 g) were soaked in sufficient amount of methanol for one week at room temperature with occasional shaking and stirring then filtered through a cotton plug followed by Whitman filter paper No. 1. The solvent was evaporated under vacuum at room temperature to yield semisolid. The extract was then preserved in a refrigerator till further use.

2.3 Chemicals and drugs

The chemicals used were Bovine serum albumin (BSA), Diclofenac sodium were purchased from Sigma-Aldrich. All chemicals in this investigation were of analytical reagent grade.

3. *In vitro* anti-arthritic activity

For the assesment of *in vitro* anti-arthritic activity of *B. retusa* and *A. hookerianum*, the method used was "inhibition of protein denaturation" [6-9] using diclofenac sodium a standard. The test solution (0.5 ml) consists of 0.45 ml of bovine serum albumin (5% w/v aqueous solution) and 0.05 ml of test solution (methanolic extract of *B. retusa* and *A. hookerianum*). The test control solution (0.5 ml) consists of 0.45 ml of bovine serum albumin (5% w/v aqueous solution) and 0.05 ml of distilled water. Product control (0.5 ml) consists of 0.45 ml of distilled water and 0.05 ml of test solution. Standard solution (0.5 ml) consists of 0.45 ml of bovine serum albumin (5% w/v aqueous solution) and 0.05 ml of diclofenac sodium. Various concentrations (31.25, 62.5, 125, 250, 500, 1000 µg/ml) of methanolic extract of *B. retusa*, *A. hookerianum* and diclofenac sodium (standard) were taken, respectively. All the solutions were adjusted to pH 6.3 using 1 N HCl. Samples were incubated at 37 °C for 20 min and the temperature was increased to keep the samples at 57 °C for 3 min. After cooling, 2.5 ml of phosphate buffer was added to the previous solutions. The absorbance was measured using UV-Visible spectrophotometer at 416 nm. The control represents 100% protein denaturation. The results were compared with diclofenac sodium. The percentage inhibition of protein denaturation of different concentrations is tabulated in Table 1. The percentage inhibition of protein denaturation can be calculated as:

$$\% \text{ of Inhibition} = [100 - (\text{OD of test solution} - \text{OD of product control})] \times 100$$

Where OD = optical density.

The control represents 100% protein denaturation. The results were compared with diclofenac sodium.

4. Results

4.1 Anti-arthritic study

Different concentrations of methanol extract of *Breynia retusa*, *Aglaonema hookerianum* and diclofenac sodium were tested for anti-arthritic activity and found significant percentage inhibition in protein denaturation (Table 1). Here, in lower concentration methanolic extract of *Breynia retusa* and *Aglaonema hookerianum* showed 33.87% and 43.54%, where the standard drug diclofenac sodium showed 52.15% of inhibition. And in higher concentration, the extract of *Breynia retusa* and *Aglaonema hookerianum* exhibited the 75.86% and 81.43% of inhibition, in where the diclofenac sodium exhibited 92.77% of inhibition of protein denaturation.

Table 1: Percent inhibition of protein denaturation of *Breynia retusa* and *Aglaonema hookerianum*

Concentration (µg/ml)	Percent of inhibiton in protein denaturation		
	BR (Test Solution)	AH (Test Solution)	Diclofenac sodium
31.25	33.87	43.54	52.15
62.5	37.09	50.02	61.82
125	40.32	56.45	64.51
250	43.54	58.06	83.33
500	62.90	69.35	86.02
1000	75.86	81.43	92.77

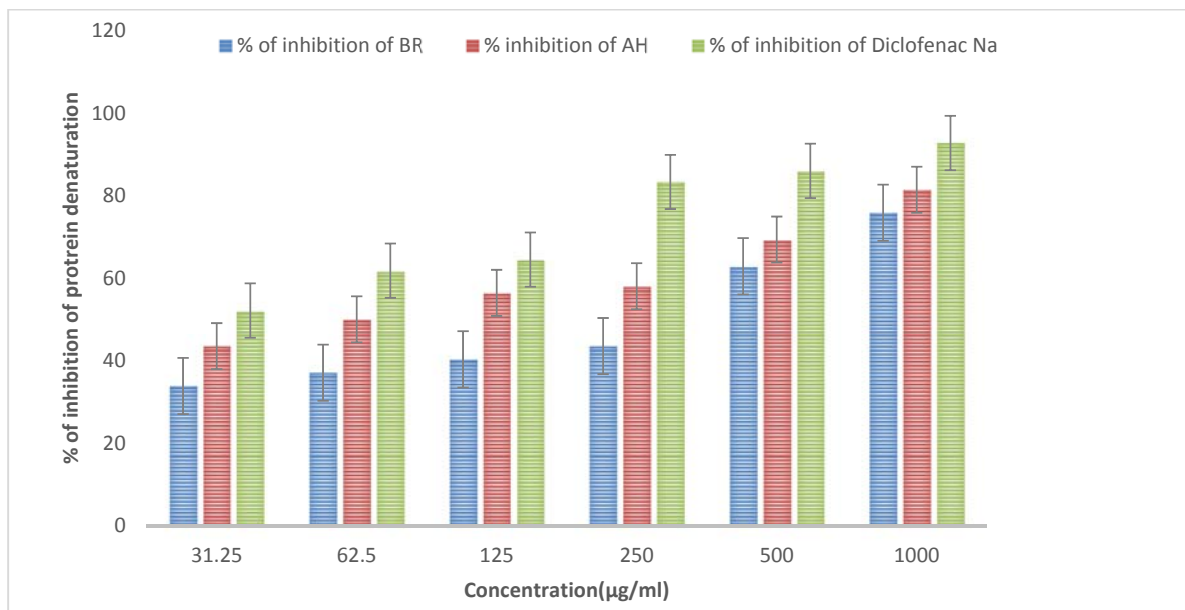


Fig 1: Graphical comparison of *percentage of inhibition* of methanolic extract of *Breynia retusa* and *Aglaonema hookerianum* leaves and the standard diclofenac sodium on protein denaturation

5. Discussion

Arthritis is a type of joint disorder that involves inflammation of one or more joints, accountable for pain swelling, stiffness, loss of function in joint. One of the main reason of the arthritis is denaturation of protein. In certain arthritic disease, auto antigen is produced due to the denaturation of protein. The

mechanism of denaturation is probably involved in the alteration of electrostatic hydrogen, hydrophobic and disulphide bonding [10]. In this study, methanolic extracts of both plants have shown significant activity at various concentrations and the effects were compared with the standard drug diclofenac sodium. The maximum percentage

inhibition of protein denaturation of *Breynia retusa* and *Aglaonema hookerianum* were observed as 75.86% and 81.43% at 1000 µg/ml respectively which were close to the percentage of inhibition of diclofenac sodium (92.77%). From this result, it can be stated that these extracts are capable of controlling the production of auto antigen to inhibit the denaturation of protein. According to this studies, it could be concluded that *A. hookerianum* (Araceae) have maximum anti-arthritis activity than *B. retusa* (Euphorbiaceae) and *A. hookerianum* could be used as natural anti-arthritic source and thus could be useful as curative agents in impeding the diseases. Further studies are needed for their active principle to illuminate.

6. Conflict of interest statement

We declare that we have no conflict of interest.

7. Acknowledgement

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